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COMPARISON OF TWO ESTROUS SYNCHRONIZATION PROTOCOLS ON REPRODUCTIVE PERFORMANCE OF LACTATING DAIRY COWS

By

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This Thesis was Submitted in Partial Fulfillment of the Requirements for the Master's Degree of Science in Animal Production

Faculty of Graduate Studies

The University of Jordan

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This Thesis (Comparison of Two Estrous Synchronization Protocols on Reproductive Performance of Lactating Dairy Cows) was successfully Defended and Approved on July 28, 2011

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DEDICATION

I dedicate this achievement to my family. To the spirit of my mother, to my father, uncle, wife, brothers and sister, and to my stepmother, their love, support, and guidance represent a source of inspiration.

Finally, I dedicate this achievement also to my friends; their support and encouragement has been essential.

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AKNOWLEDGEMENT

I would like to express my thanks and appreciations to all who teach and support me through my whole life. Thanks are to Dr. Mufeed Alnimer (my supervisor) for his patience, guidance, and continuous support throughout my research, to the examination committee represented by Dr. Abdur-Rahman Al-Fataftah, Mohammad Jalal, and Dr. Rami Kridli, and to the academic staff in the department of animal production in the University of Jordan.

My deep thanks and appreciations are also to the Jordan Union for Agricultural and Zoological Investment for providing its animals and facilities and all workers in the farm for their assistance.

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LIST OF ABBREVIATIONS

Abbreviation	Word (s)
AI	Artificial Insemination
CL	Corpus Luteum
CP	Crude Protein
CV %	Coefficient of Variation
DMI	Dry Matter Intake
E_2	Estradiol-$_{17\beta}$
EB	Estradiol Benzoate
ECP	Estradiol Cypionate
EP	Estradiol Propionate
FSH	Follicle-Stimulating Hormone
GnRH	Gonadotrophin Releasing Hormone
hCG	human Chorionic Gonadotrophin
IGF-1	Insulin-Like Growth Factor-1
LH	Luteinizing Hormone
NEB	Negative Energy Balance
NE_L	Net Energy of Lactation
NRC	National Research Council
P_4	Progesterone
PGE_2	Prostaglandin E_2
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
pp	Postpartum
RIA	Radioimmunoassay
Short ES	Short Estrus Synchronization
TAI	Timed Artificial Insemination
TMR	Total Mixed Ration

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ABSTRACT

Fifty-three Holstein Friesian lactating dairy cows were used to evaluate the effect of two-estrus synchronization protocols starting between d 10-12 of the estrous cycle on reproductive performance. Ovsynch group (Control, n=24): cows received the first GnRH injection on day 46 postpartum (pp) and $PGF_{2\alpha}$ 7 d later, and then the second GnRH injection two days later and artificial insemination (AI) was performed between 16-20 h from the last GnRH injection. Short ES group (n=29):- cows received $PGF_{2\alpha}$ injection on day 46 pp then E_2 injection after 48 h and GnRH injection 12 h after E_2 followed by AI at detected estrus. Cows in both groups were subjected to an estrus detection program by visual observation. Blood samples were collected from all cows at days 0 (day of AI), 7, 14, 21, 22, and 23 after AI to determine P_4 concentrations. Pregnancy rates from first AI were based on plasma P_4 concentrations on d 22±1 post AI, and confirmed by rectal palpation between days 45 and 50 post AI. Results showed that more cows ($P<0.01$) in the Short ES group (86.2%) exhibited estrus than in the Ovsynch group (41.7%). Pregnancy rates to first AI were greater ($P < 0.05$) in the Short ES group (37.4%) than in the Ovsynch (12.5%) group. In contrast, pregnancy losses were lower ($P < 0.05$) in the Short ES group (38.9%) than in the Ovsynch (76.9%) group. Pregnancy rates to second, third, and overall and pregnancy losses were similar between both groups and between primiparous and multiparous cows. Days open for pregnant cows tended to be higher ($P<0.09$) in the Ovsynch group than in the Short ES group. Plasma P_4 concentrations were similar between pregnant and nonpregnant cows on days 0 and 7 after AI, however, were greater ($P<0.01$) for pregnant than nonpregnant cows on days 14, 21, 22, and 23 after AI. In conclusion, Short ES protocol improves reproductive performance by increasing the number of cows exhibiting estrus and pregnancy rates after the first AI compared with Ovsynch protocol.

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1. INTRODUCTION

Increasing demand for milk production has led to a decline in the reproductive performance of dairy cows because of a prolonged calving interval (Butler, 2000). Several factors such as longer time period from parturition to first estrus, poor estrus expression or detection, improper timing of artificial insemination (AI), and reduction in conception rates at first AI have contributed to a longer calving interval, and in doing so, have compromised profitability in dairy farming (Sturman, et al., 2000). The situation is further complicated by the fact that high yielding early postpartum dairy cows do not often develop regular ovarian activity (Opsomer, et al., 2000).

Synchronization of estrus implies the manipulation of the estrous cycle or induction of estrus to bring a large percentage of a group of cows into estrus at a predetermined time (Odde, 1990). For this purpose, various synchronization programs allowing timed artificial insemination (TAI) have been developed for cows. Synchronization of ovulation (Ovsynch) was the first protocol developed that synchronizes ovulation within a period of 8 hours (Pursley, et al., 1995, 1997a, b). This protocol involves two injections of gonadotrophin releasing hormone (GnRH) 7 d before and 48 h after a prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) injection with timed AI after 16h of the second GnRH. This protocol has been shown to improve pregnancy rates in several studies (Cartmill, et al., 2001; Moreira, et al., 2001; Lubbadeh and Alnimer, 2003; El-Zarkouny, et al., 2004). In Jordan, pregnancy rates at first AI after Ovsynch application range from about 20 to 40% (Lubbadeh and Alnimer, 2003; Alnimer, 2005a, b). However, this protocol requires several days to sort cows and perform the treatment, and it is difficult to reduce the interval between the start of treatment and AI to less than 9 days (Lopes, et al., 2000).

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Estradiol-$_{17\beta}$ (E_2) was included in estrus synchronization programs as an alternative to the second GnRH or as an additional hormonal treatment in the Ovsynch protocol. Most studies (Pancarci, et al., 2002; Cerri, et al., 2004; Alnimer, 2005a; Souza, et al., 2007, Hillegass, et al., 2008; Brusveen, et al, 2009) found no effect of using of the E_2 in the Ovsynch protocol on pregnancy rates. Previous studies demonstrated that administration of a luteolytic dose of $PGF_{2\alpha}$ followed by human Chorionic Gonadotrophin (hCG) plus estradiol synchronized estrus in all cows in the luteal phase produces a short synchronization program (Lopez - Gatius, 2000).

A short method for estrus synchronization in dairy cows to save time by decreasing the interval to AI was developed by Cirit, et al. (2008a). It involves injection of $PGF_{2\alpha}$ and then injection of E_2 after 48 h then GnRH injection after 12 h followed by TAI within 16-20 h after GnRH. This method needs a functional corpus luteum (CL) to succeed. They reported that estrus detection rate, ovulation synchronization rate and pregnancy rates for treated cows were 55, 94.1 and 35%, respectively. In addition, the same hormonal treatments ($PGF_{2\alpha}$, Estradiol Cypionate (ECP), and GnRH) were applied to another group of cows but at fixed interval between them (24 h). The protocol resulted in 59.4% estrus expression, 59.1% ovulation synchronization rate, and 31.3% pregnancy rates. However, Cirit et al. (2008a) did not compare the short method of synchronization with the Ovsynch protocol. Therefore, the objective of this study was to test the hypothesis that using short estrus synchronization method in the middle of estrous cycle with AI after estrus detection in dairy cows would improve reproductive performance compared to the Ovsynch protocol.

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2. LITERATURE REVIEW

2.1. Reproductive Performance

2.1.1. After Calving

After calving, the reproductive strategy of the dairy cow changes from nourishing and delivering a healthy calf to re-establishing pregnancy (Rhodes, et al., 2003). The dairy cow enters anovulatory anestrous period following parturition, and needs typically between 19-22 d from calving to first ovulation (Rhodes, et al., 2003). The duration of postpartum (pp) anestrus is affected by several environmental, genetic, physiologic, and metabolic factors. Ninety percent of cows have their first pp ovulation within 6 weeks after calving, however, the interval from calving to ovulation can be 3 weeks longer in the present dairy cows (Peter, et al., 2009). The duration of pp anestrus is not determined by emergence of follicular waves, but rather by follicular deviation and/or the fate of the dominant follicle. Under optimal conditions, there is deviation in follicular growth, selection of a dominant follicle, its growth to maturity, ovulation, and subsequently luteolysis, resulting in reestablishment of cyclical ovarian activity, with an opportunity for becoming pregnant. Failure of any of these processes prolongs pp anestrus. In addition, failure of cows to express estrus and poor estrus detection efficiency can increase the incidence of pp anestrus in a dairy herd (Peter, et al., 2009).

2.1.2. Ovarian Activity

Energy balance during the first 20 days of lactation is important in determining the onset of pp ovarian activity while the time required for pp uterine involution varies from 4 to 6 weeks. Subclinical and clinical postpartum uterine infections affect ovarian activity. It has been hypothesized that uterine diseases suppress hypothalamic gonadotropin-releasing hormone (GnRH) and possibly pituitary luteinizing hormone

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(LH) secretion and have localized effects (i.e., delaying folliculongenesis) on ovarian function.

Due to the strong negative feedback from progesterone (P_4) and E_2, concentrations of gonadotrophic hormones are very low in late pregnancy (Rhodes, et al., 2003). Within 5-10 d after calving, concentrations of follicle stimulating hormone (FSH) increase, thus initiating the growth and development of ovarian follicles. A single follicle becomes the dominant follicle after around 4 d of FSH increase (Rhodes, et al., 2003). This first dominant follicle may ovulate by responding to LH surge due to the sufficient E_2 production by the follicle. In contrast, the dominant follicle may become atretic or cystic because the E_2 production from that follicle is insufficient to induce LH surge necessary for ovulation due to inadequate stimulation of LH pulse frequency, insulin-like growth factor-1 (IGF-1), and Insulin. Both IGF-1 and Insulin are needed for the proliferation of granulosa and theca cells that are responsible for steroidogenesis. However, the first pp ovulation is generally associated with the absence of estrus behaviour and is followed by a short luteal phase due to the premature release of Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the uterus (Rhodes, et al., 2003). Frequent occurrences of estrus following parturition help in pp fertility improvement. Cows that express estrus more than one time pp had greater fertility than those that have no or one pp estrus (Thatcher, et al., 2006; Friggens and Labouriau, 2010).

2.1.3. First Service

In lactating dairy cows, the interval from parturition to first ovulation is related to the level of milk production and is longer in cows with a higher genetic potential for milk yield. It was reported that cows with high 305-d milk yield had lower reproductive efficiency than those with low 305-d milk yield including long interval from calving to first service and to conception, high number of services per conception, first service

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conception rate, and overall conception rate (Dhaliwal, et al., 1996). About half of the 305-d yield is produced in the first third of lactation in which the peak milk yield is achieved between 40 and 75 d. This means that the recommended calving to first service interval of 60 d may coincide with the periodic peak lactation (Dhaliwal, et al., 1996). However, with the increase in the calving to first service interval (i. e. when the peak lactation was over), the fertility of high - yielding cows was improved rapidly than in the low yielding cows (Dhaliwal, et al., 1996). In addition to that, Wathes et al. (2007) reported that multiparous not primiparous cows were affected by peak milk yield. Wathes et al. (2007) found that multiparous cows that took more than 150 d from calving to conception produced an average of 13 kg/d more milk in the peak lactation period than those conceiving in less than 80 d, while no relation was found between peak lactation period and interval from calving to conception in primiparous cows.

Following parturition, dry matter intake (DMI) needs to increase about 4-6 times in order to meet the high nutrient demands of milk production. On the other hand, the high yielding dairy cow after calving is unable to increase its DMI rapidly to satisfy demands for lactation; as is dependent on its body reserves of fat and protein (Roche, et al., 2000). This scenario places the cow in a negative energy balance (NEB) state in which body stores (mainly fat) are used for lactation, maintenance, and growth while the reproductive tract receives the lowest supplement (Mwaanga and Janowski, 2000; Peter, et al., 2009). The NEB affects reproduction through a decrease in LH pulse frequency, a decrease in the diameter of the dominant follicle with low E_2 production, a decrease in CL size with low P_4 production, a decrease in IGF-1 availability, and an increase the interval to first pp estrus (Roche, et al., 2000).

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2.1.4. Estrus Detection

The major factor limiting reproductive performance on many dairy farms is the failure to detect estrus in a timely and accurate manner. Efficient and accurate estrus detection is essential to optimize the economic management of individual cows to yield a profitable dairy operation through obtaining higher pregnancy rates (Roche, et al., 2000). The failure to detect cows in estrus accurately is the main reason for increasing the calving interval in many dairy herds. Estrus detection rates rarely reach more than 50%, and not all cows inseminated are identified accurately as being in estrus, which results in lower conception rates (Mialot, et al; 1999). Estrus synchronization programs aim to correct this difficulty in estrus detection and AI accuracy. However, despite progress in synchronizing estrus, the reproductive performance of dairy cows has not substantially improved (Lopez - Gatius, 2000).

Calving interval is a major component and is primarily a function of the number of days from calving to the initiation of the next pregnancy, referred to as days open, and the fixed effect of gestation length. The days open depend on the days from calving to the first insemination or mating, and are associated with conception rate, along with the interval to conception, and conception rate at repeat breeding. A calving interval of 12 to 13 months is generally considered economically optimal for dairy cows (Larson and Ball, 1992).

2.1.5. Milk Production

Reproduction is important for sustainable dairying worldwide. It is one of the key components of profitable dairy systems besides milk production and disease, however, the high producing dairy cows of the recent years are subfertile (Grohn and Rajala-Schultz, 2000; Thatcher, et al., 2006).

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Under the current production systems, modern high producing dairy cows have low reproductive efficiency (Butler, 2003; Chebel, et al., 2004; Thatcher, et al., 2006; Wiltbank, et al., 2006). Moreover, the reproductive efficiency of the dairy cows has decreased in the past two decades with an increase in milk yield, which is related to the prolonged NEB (Grohn and Rajala-Schultz, 2000; Roche, et al., 2000, Washburn, et al., 2002). Based on the analyses of large data sets, there is clearly an antagonistic relationship between milk yield and reproduction in dairy cattle (Lucy, 2001). Increased genetic capability for milk yield coupled with changes in nutritional management and larger herd size have been associated with the decline in fertility of lactating cows. Epidemiological studies indicate that other factors such as reproductive diseases (i.e., retained placenta, metritis and ovarian cysts) or season of calving were relatively more important than milk yield on influencing reproductive performance. However, it is clear that lactation, as a physiological process, is associated with a lower reproductive rate compared to that of non-lactating heifers (Thatcher et al., 2006). According to Sturman, et al. (2000), the management of the herd for high reproductive efficiency is an important economic factor in the success of a dairy enterprise. To maintain competitiveness, dairy farms have increased average herd size and maximized milk yield. These increases have been implicated as contributors to a lower reproductive efficiency experienced by many dairy farms because reproductive performance appears to be directly related to herd management (Nebel and Jobst, 1998)

2.1.6. Heat Stress

It is well documented that heat stress contributes in reproductive failure of dairy cows (Wolfenson, et al., 2000; De Rensis and Scaramuzzi, 2003; Alnimer, et al., 2009). When coupled with high milk production, the problem of heat stress is exacerbated (Wolfenson, et al., 2000; Wiltbank, et al., 2006). Heat stress negatively affect

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reproductive performance of dairy cows through an alteration in follicular development and oocyte competence, steroidogenic capacity, P_4 production from CL, gonadotrophins secretion, and embryo development and quality (Badinga, et al., 1994; Wolfenson, et al., 1995; 2000; Roth, et al., 2001a & b; Al-Katanani, et al., 2002; De Rensis and Scaramuzzi, 2003).

Heat - stressed cows have a lower blood flow to the uterus and an increase in uterine temperature that inhibits embryo development leading to severe embryonic losses and failure of fertilization (Rivera and Hansen, 2001). However, the embryo seems to be sensitive to heat stress in the early stages of development (less than 3 d) because it is unable to produce protective molecules (Drost, et al., 1999). Once the pregnancy diagnosis show a positive result at day 31 post AI, subsequent exposure to heat stress will be of little or no effect on embryo viability at least for the following 14 d (Chebel, et al., 2004).

In California (USA), a study by Santos et al. (2009) reported lesser pregnancy rates for cows inseminated in summer (30.3%) and autumn (29.8%) months compared to cows inseminated in winter (33.7%) and spring (35.3%) months. In Jordan, Alnimer et al. (2009) found greater pregnancy rates and lower pregnancy losses for cows inseminated in winter (39.3 and 8.5%; respectively) compared to those inseminated in summer (18.9 and 40.0 %; respectively).

Since around 42 days (two estrous cycles) are needed for the follicle to grow from 0.13 mm to the preovulatory size (Lussier, et al., 1987), the negative effects of summer heat stress on reproductive efficiency are carried over through autumn months (Roth et al., 2001a & b). A study by Chebel et al. (2004) found lower pregnancy rates for cows exposed to heat stress 20 to 50 d before TAI.

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Pregnancy losses also have their negative effects on reproduction. Most pregnancy losses (around 30%) occur during the early embryonic period (before 24 d post AI) and late embryonic period (24-45 d post AI) (Santos, et al., 2004; Starbuck, et al., 2004; Gabor, et al., 2008). According to Santos et al. (2004), fertilization rate in lactating dairy cows averaged 76% with around 50% embryo viability on day 6 post insemination, and approximately 35% conception rate at the end of the embryonic period.

Humblot (2001) found that pregnancy losses were greater in the early embryonic period (31.6%) than in the late embryonic period (14.7%). Moreover, Santos et al. (2004) reported a rate of 0.85% pregnancy losses per day (12.8%) between day 30 and 45 post AI. Chebel et al. (2004) found similar results at the same period (7.3% to 15.3%). However, late embryonic losses ranged from 3.2 % in lower producing cows (Silke, et al., 2002) and 42.7% in high producing cows exposed to heat stress (Cartmill, et al., 2001).

It was reported that extended periods of follicle dominance affect oocyte quality and subsequent embryo viability (Santos, et al., 2004; Cerri, et al., 2009). Follicular dominance should be restricted to 5-6 days and any small increase in the length of dominance can cause an increase in number of degenerated oocytes and embryos (Cerri, et al., 2009).

Lower concentrations of P_4 in milk and plasma between days 10 and 12 following insemination were reported as a main factor contributing to pregnancy failure (Mann and Lamming, 1999). These lower concentrations of P_4 could be due to enhanced luteolysis or antiluteotropic actions within the CL (Starbuck, et al., 2004; and Rhinehart, et al., 2009). However, Rhinehart et al., (2009) concluded that low serum concentrations of P_4 in lactating dairy cows susceptible to pregnancy failure are likely to be the result of increased clearance of P_4 rather than decreased luteal production. In addition,

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Wiltbank et al. (2006) reported that increased metabolism by the liver decreases serum concentrations of P_4 during the estrous cycle of lactating dairy cows.

Milk yield was reported as an important factor that contributes to an increase in pregnancy losses. Grimard et al. (2006) found greater pregnancy losses for high - producing compared to low - producing dairy cows. In addition, primiparous cows that were generally accepted as lower producing cows had lower pregnancy losses than multiparous cows (Chebel, et al., 2004; Alnimer, et al., 2009; Santos, et al., 2009).

2.2. Estrous Cycle

The bovine female is polyestrous with an average estrous cycle length of approximately 21 d (Sailisbury, et al., 1978; Peters and Ball 1995; Bearden and fuqay, 1997). There are four stages to the estrous cycle, each with unique hormonal and ovarian characteristics (Bearden and Fuqay, 1997). These stages are:

2.2.1. Estrus

Estrus is the period during which a cow or heifer displays sexual behavior and allows mounting by herd mates or a bull. This period can last from a few hours to as long as 24 h, with 10 to 12 h being the normal range. Other sexual behaviors exhibited include head butting, mounting herd mates or the bull, vocalization, mucus discharge, and general hyperactivity may last for 16 to 24 h (Salisbury, et al., 1978; Roelofs, 2005). Hormonally, the female is under the influence of increased circulating concentrations of the ovarian steroid E_2, which peaks approximately 24 h before the onset of estrus (Glencross and Pope, 1981). The onset of estrus is associated with increased serum concentrations of FSH and a dramatic pre-ovulatory peak, or surge, of LH (Chenault, et al., 1975; Bernard, et al, 1984).

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2.2.2. Metestrus

The period from approximately d 1 to 5 following estrus is termed metestrus. This stage is associated with a sharp decline in estradiol, LH, and FSH concurrent with a steady increase in P_4 secreted from the developing CL (Chenault, et al., 1975; Salisbury, et al., 1978; Bearden and Fuquay, 1997). Intervals from onset of estrus to ovulation in *Bos taurus* cattle have been reported as 30 h (Roelofs, et al., 2005) and 27 h (Bernard, et al., 1984), or approximately 14 h after the end of estrus (Bearden and Fuquay, 1997). The average interval to ovulation from the onset of estrus was 25 h with a range of 13 to 34 h. Following ovulation, the animal is no longer sexually receptive, but may appear mildly restless and show a slight bloody discharge due to the rupture of uterine capillaries referred to as metestrus bleeding (Salisbury, et al., 1978; Bearden and Fuqay, 1997).

2.2.3. Diestrus

The third stage of the bovine estrous cycle is termed diestrus and corresponds to the luteal phase (Peters and Ball, 1995; Senger, 2003). During this stage, from approximately d 5 to d 17 of the cycle (Bearden and Fuqay, 1997), the bovine female has increasing concentrations of circulating P_4 produced by the CL, with peak concentrations reached around d 17 (Vaca, et al., 1983). During diestrus, serum LH is suppressed by the increased circulating concentrations of P_4 (Hafez and Hafez, 2000). It is during this stage that maternal recognition of pregnancy occurs. The bovine conceptus, similar to ovine and caprine conceptuses, secretes interferon tau which is the signal for maternal recognition of pregnancy (Roberts, et al., 1992; Bazer, et al ., 1994). The critical time for maternal recognition in the cow is between d 15 and 16 of the cycle (Bazer, et al., 1994).

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2.2.4. Proestrus

The final stage of the estrous cycle is proestrus, more commonly referred to as the follicular phase. The follicular phase is characterized by sharply declining serum concentrations of P_4 due to lysis of the CL by $PGF_{2\alpha}$ released from the endometrium of the uterus (Peters and Ball, 1995; McCracken, et al., 1999; Curtis-Prior, 2004). From approximately d 17 to ovulation the animal experiences rising serum concentrations of FSH, LH and E_2 in preparation for ovulation (Ginther et al., 1997; Senger, 2003).

2.3. Systems Used to Synchronize First Insemination

2.3.1. Injection of Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)

Prostaglandins, mainly $PGF_{2\alpha}$ and prostaglandin E_2 (PGE_2), are 20-carbon unsaturated hydroxyl fatty acids with a cyclopentene ring derived from the arachidonic essential fatty acid. Most prostaglandins act locally at the site of their production on a cell-to-cell interaction, and therefore, do not conform exactly to the classic definition of a hormone unlike other humoral agents. Prostaglandins are not localized in any particular tissue; they are transported in the blood to act on a target tissue away from the site of production. Some forms never appear in the blood, whereas others are degraded after they circulate throughout the liver and lungs. $PGF_{2\alpha}$ is the natural luteolytic agent that ends the luteal phase (CL life) of the estrous cycle and allows for the initiation of a new estrous cycle in the absence of fertilization. It is particularly potent in ending early pregnancy (Hafez and Hafez, 2000).

A double protocol in which $PGF_{2\alpha}$ was given at a 7, 11 or 14-day interval was developed so that cows would be at a stage in the estrous cycle (diestrus) with a fully functional CL when they received the second $PGF_{2\alpha}$ dose. The 14-day interval double $PGF_{2\alpha}$ regimen seems to show an improved response over the 7-and 11-day protocol, as it ensures that most animals are in the late luteal stage (cycle days 11-14) when they

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receive the second $PGF_{2\alpha}$ dose (Stevenson, et al., 1999; Folman, et al., 1990; Ferguson and Gilligan, 1993).

The injection of $PGF_{2\alpha}$ will cause immediate regression of the CL after day 5 of the estrous cycle of cattle, thus causing a rapid decline in P_4 concentrations to basal concentrations within 24 h. Therefore, LH pulse frequency increases, causing a significant increase in E_2 from the dominant follicle and induction of estrus and ovulation. Despite this rapid luteolysis, the interval to onset of estrus is variable and is dependent on the stage of the follicle wave the animal is at when treated. Animals with a functional dominant follicle will be in estrus in 2-3 day because the dominant follicle at the time of induced luteolysis ovulates (Savio, et al., 1988).

2.3.2. Gonadotrophin-Releasing Hormone (GnRH) and $PGF_{2\alpha}$

Gonadotrophin-Releasing Hormone is a neuropeptide hormone of hypothalamic origin that controls LH and FSH secretions of the anterior pituitary gland (Schneider, et al., 2006). The injection of GnRH i.m. stimulates the anterior pituitary gland to secrete LH and FSH necessary for follicular development within 2-4 h. Luteinizing hormone and FSH act by binding to their receptors on the luteal and follicular cells, respectively.

Synchronization programs must synchronize ovarian follicular dynamics and CL regression (Thatcher, et al., 1989). To apply this, a program involving GnRH and $PGF_{2\alpha}$ was developed to concentrate the estrus activity within a limited period. Thatcher et al. (1989), Wolfenson et al. (1994), and Stevenson et al. (1999) found that this program allowed cows to be in estrus 3-5 d earlier than those treated with $PGF_{2\alpha}$ alone did. In addition, Stevenson et al. (1996) and Jobst et al. (2000) found no differences in pregnancy rates between programs based on GnRH + $PGF_{2\alpha}$ or those based on $PGF_{2\alpha}$ alone.

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2.3.3. Synchronization of Ovulation (Ovsynch)

The Ovsynch protocol was developed by Pursley et al. (1995) to synchronize ovulation within a certain time. Using Ovsynch as a reproductive management tool is beneficial because reliance on estrus detection for breeding cows can be minimized. This protocol eliminates the need for estrus detection and provides a 100% submission rate. Ovsynch commences with an injection of GnRH that can be given to lactating dairy cows at any stage of the estrous cycle. Seven days later, an injection of $PGF_{2\alpha}$ is given followed by a second injection of GnRH two days later. Cows then receive a timed AI between 16 and 24 hours after the second GnRH injection without regard to estrus behavior (Pursley, et al., 1995, 1997a & b). The first GnRH injection aims to cause either ovulation or luteinization of the dominant follicle and prevents estrus until after $PGF_{2\alpha}$ injection 7 d later that causes the CL or luteinized follicle to regress. A new dominant follicle forms and is available to be ovulated by the second GnRH injection given 48 h after $PGF_{2\alpha}$, followed by the timed AI after 16 h of the second GnRH injection (Pursley, et al., 1995; 1997a & b).

The Ovsynch success depends on the response to the first GnRH injection, which varies according to the stage of the estrous cycle and follicular development at the time of treatment. Different studies have reported a range between 54 and 90% ovulation rate to the first GnRH (Pursley, et al., 1995; Vasconcelos, et al., 1999; Bello, et al., 2006).

Bello et al. (2006) concluded that interwave intervals (time from onset of one follicle wave emergence to another) are a physiological factor that could affect the response to the first GnRH injection. If it is longer, the ovulation probability to the first GnRH injected randomly increases because fewer cows are in the first stages of the folliculur wave. In contrast, if it is shorter, the ovulation probability decreases due to greater percentage of cows being in early follicular development phase.

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The efficiency of $PGF_{2\alpha}$ injection depends primarily on the response of cows to the first GnRH injection. Failure of luteolysis synchronization to $PGF_{2\alpha}$ was explained through CL regression before $PGF_{2\alpha}$ or failure to respond to $PGF_{2\alpha}$ (Bello et al., 2006). Cows that showed estrus before $PGF_{2\alpha}$ injection were more likely to have regressed CL and had a spontaneous preovulatory LH surge before the second GnRH (Pursley, et al., 1997b; Vasconcelos, et al., 1999; Moreira, et al., 2000).

The second injection of GnRH has little flexibility ranging from 36-48 h after $PGF_{2\alpha}$ (Schmitt, et al., 1996; Peters and Pursley, 2003). Pursley et al. (1995) reported that this protocol synchronizes ovulations within 24 to 32 h after the second GnRH injection.

Across large numbers of previous studies, pregnancy rates to Ovsynch generally ranged between 30 and 40%, which is similar to conception rates to AI after a detected estrus (Burke, et al., 1996; Pursley, et al., 1997a & b; Stevenson, et al., 1999; Alnimer et al., 2002; Lubbadeh and Alnimer, 2003).

In addition, primiparous cows were found to have greater pregnancy rates than multiparous cows (Cartmill, et al., 2001; Chebel, et al., 2004; Tenhagen, et al., 2004; Alnimer, et al., 2009) due to high metabolic disorders (especially NEB) and reproductive problems in multiparous than in primiparous cows (Tenhagen, et al., 2004).

2.3.4. Presynchronization – Ovsynch Protocol

Based on the idea that the day at which the Ovsynch protocol starts resulted in different rates of ovulation to the first GnRH injection, synchronization to the second GnRH, and pregnancy rates; the Presynchronization (Presynch) program was applied prior to the implementation of the Ovsynch protocol. About 96% of cows ovulated when Ovsynch was started between day 5 and 9 of the estrous cycle (Vasconcelos, et al.,

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1999). Therefore, the initiation of the Ovsynch protocol between day 5 and 12 of the estrous cycle produced more pregnancy rates in cyclic dairy cows (Vasconcelos, et al., 1999; Cartmill, et al., 2001; Moreira, et al., 2001). The Presynch program involves two injections of $PGF_{2\alpha}$ 14 d apart with the second one 11 to 14 d before the first GnRH of the Ovsynch protocol (Moreira, et al., 2001; El-Zarkouny, et al., 2004; Navanukraw, et al., 2004; Galvao, et al., 2007). Using the Presynch program before Ovsynch increases the number of cows in early diestrus at the beginning of the Ovsynch and consequently produced higher pregnancy rates (Moreira, et al., 2001; El-Zarkouny, et al., 2004). Moreover, a greater percentage of cows was classified as "High-High" (P_4 concentrations were high at first GnRH and $PGF_{2\alpha}$ of the Ovsynch) following Presynch compared with cows not presynchronized (Moreira, et al., 2001; El-Zarkouny, et al., 2004).

Most studies used different intervals of 11 to 14 d between the $PGF_{2\alpha}$ injection of the presynch and GnRH injection of the Ovsynch protocol and reported an improvement in pregnancy rates compared to the Ovsynch alone (Moreira, et al., 2000; 2001; El-Zarkouny, et al., 2004; Navanukraw, et al., 2004; Galvao, et al., 2007). The 14 d interval applied by Navanukraw et al. (2004) produced similar pregnancy rates to the 12 d interval used by Moreira et al. (2000 and 2001) and El-Zarkouny, et al. (2004). In contrast, Galvao, et al. (2007) found that the interval of 11 d produced greater pregnancy rates than that of 14 d.

Some researchers used a single $PGF_{2\alpha}$ injection as a presycynch program 10 or 12 d before of Ovsynch protocol is commenced (Cordoba and Fricke, 2001; LeBlanc and Lesile, 2003) and reported no advantage when compared to the Ovsynch protocol alone. The advantage of the double $PGF_{2\alpha}$ injections over the single $PGF_{2\alpha}$ injection was explained by the positive effect on the uterine environment and the number of estruses

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that occur during the pp period. This means that double $PGF_{2\alpha}$ injections targeting the Ovsynch protocol to initiate in a more favorable stage of the estrous cycle (days 5 to 12) at which the dominant follicle of the first follicular wave is ready to ovulate (Moreira, et al., 2001; Navanukraw, et al., 2004). It was postulated that as the number of estrous cycles before first service increases, conception rates to the first service also increase (Thatcher and Wilcox, 1973), and that use of $PGF_{2\alpha}$ in pp cows may induce estrus before first service and increase first-service conception rates (Young et al., 1985).

2.3.5. Estradiol Inclusion in the Ovsynch Protocol

Estradiol - $_{17\beta}$ is the biologically active estrogen produced by the ovary with smaller quantities of estrone. It is known in inducing the preovulatory- like LH surge, ovulation, and luteolytic activity during the luteal phase due to its positive feedback effect on LH (Pancarci et al., 2002; Cerri et al., 2004). In addition, high producing cows often lack sufficient $E_{17\beta}$ concentrations in blood for induction of estrus, ovulation, and uterine priming because of the extensive steroid metabolism and clearance (Wiltbank et al., 2006). These effects motivated bovine reproductive physiologists to include it in the estrus synchronization protocols.

Pancarci et al. (2002) used estradiol cypionate (ECP) instead of the second GnRH at 24 h after $PGF_{2\alpha}$ before TAI 48 h later ECP (Heatsynch protocol). The results recorded similar pregnancy rates for the Ovsynch and heatsynch protocols (37.1 and 35.1%, respectively). In addition, multiparous cows of the heatsynch protocol had lower pregnancy rates than those for primiparous cows in the Ovsynch protocol (19.4 vs. 30.6%) which referred -according to authors- to the differences in the starting day for protocols after calving.

Cerri et al. (2004) compared the heatsynch protocol to a protocol dependant on using GnRH and $PGF_{2\alpha}$ with AI at detected estrus. The results indicated that pregnancy

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rates were greater for cows treated with heatsynch protocol than for those in the estrus-detected group while pregnancy losses did not differ. In addition, pregnancy rates were greater for multiparous and primiparous cows in the heatsynch group than those for cows in the estrus-detected group. It was suggested that the improvement in pregnancy rates for cows supplemented with ECP was caused by increased exposure to estradiol during proestrus, which consequently improved oocyte fertilization or better primed the uterus for the subsequent luteal phase that is important for induction of endometrial P_4 receptors (Hillegass, et al., 2008).

Alnimer (2005a) compared the Ovsynch protocol to a protocol involved using of estradiol benzoate (EB) 24 h after $PGF_{2\alpha}$ with AI at detected estrus and reported similar pregnancy rates (34.7 and 34.1%) while pregnancy rates were greater for primiparous cows than multiparous cows (38.8 vs. 24.1%). Another study by Alnimer (2005b) replaced the first and the second GnRH of the Ovsynch protocol with EB and compared it to the Ovsynch protocol. The result of this study reported comparable pregnancy rates for both protocols (40.0 and 40.4%) and for both parities (34.7% for primiparous and 43.5% for multiparous cows).

Recently, Souza et al. (2007) added E_2 8 h before the second GnRH injection of the Ovsynch protocol and compared it to the Ovsynch protocol. Pregnancy rates and pregnancy losses were similar for both protocols while estrus expression rates were greater for cows treated with Ovsynch + E_2 than that for cows treated with Ovsynch alone (80.2 vs. 44.4%). In addition, cows that showed estrus in the Ovsynch + E_2 group had greater pregnancy rates than those that showed estrus in the Ovsynch protocol and those that did not show estrus in both groups.

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Although supplementation of lactating dairy cows with E_2 increased display of estrus primarily at 72 h after $PGF_{2\alpha}$ but it had no effect on fertility (Souza, et al., 2007; Hillegass, et al., 2008; Brusveen, et al ., 2009).

2.3.6. Short Estrus Synchronization (Short ES)

A short method for estrus synchronization in dairy cows to save time by decreasing time to AI was developed by Cirit et al. (2008a). This protocol involves injection of $PGF_{2\alpha}$ followed 48 h by an injection of Estradiol propionate (EP) and then GnRH injection 12 h later before TAI within 16-20 h after the GnRH injection. This protocol resulted in 55% estrus expression, 94.1% ovulation synchronization, and 35% pregnancy rates. In addition, Cirit et al. (2008a) applied a protocol involving the same hormonal treatments ($PGF_{2\alpha}$, ECP, and GnRH) but with a 24 h interval between them and TAI at the day of GnRH injection and reported 59.4% estrus expression, 59.1% ovulation synchronization, and 31.3% pregnancy rates. Estradiol propionate (EP) and GnRH induce an LH surge approximately 16-24 h and 2-3 h after their administration in cows, respectively, and ovulation occurred 24-32 h after LH surge (Bo, et al., 1994). This may explain the short method of estrus synchronization at different intervals had higher ovulation synchronization rate and numerical pregnancy rate than that method at equal intervals.

Cirit et al. (2008a) compared between three Short ES protocols starts at different stages of estrous cycle (metestrus, diestrus, and proestrus). They reported higher estrus expression (100%) and higher pregnancy rates (62.5%) for those cows started at day 18 of ovulation (proestrus) than for those started at day 3 of ovulation (metestrus; 22.2 and 0.0%) while cows started at day 12 of ovulation (diestrus) did not differ (66.7 and 33.3%).

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Another study by Cirit et al. (2008b) applied the same Short ES method at equal intervals (24 h) but with TAI 16-20 h after the GnRH or its alternative human Chorionic Gonadotrophin (hCG). They reported comparable estrus incidence (75.8 and 70.0%) and pregnancy rates (33.3 and 40.0%) for the GnRH- and hCG- treated groups with greater synchronization ovulation rate for the hCG-treated group than GnRH-treated one (56.7% vs. 27.3%).

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3. MATERIALS and METHODS

3.1. Cows, Housing, and Management

This experiment was conducted from February to September 2009 on a commercial dairy farm (Jordan Union for Agricultural Zoological Investment) housing approximately 1600 Friesian lactating dairy cows located in the Al-Hallabat area of the northern of Jordan.

Cows were housed in a free-stall barns provided with shades and were milked three times daily at 8-h intervals. The rolling herd average milk production was approximately 9000 kg per lactation cycle. Cows were fed a total mixed ration (TMR) of 42% forage (corn silage, alfalfa hay, and straw) and 58% concentrate (corn, barley, wheat bran, soybean meal, and commercial concentrate for lactation with trace minerals and vitamins). The ration contained 1.7 Mcal net energy for lactation (NE_L)/kg, 17% crude protein (CP) (dry matter (DM) basis) and changed according to National Research Council (NRC) recommendations (2001). Cows had free access to fresh water. Weather data during the experimental period were obtained from the Official National weather Station in Alkhaldia area (Figure1). Data included maximum, minimum temperature and relative humidity with the mean (29.2 ± 2, 12.8 ± 0.15 ºC and 55.2%, respectively) and range (9.0 - 41.4, 6.0 – 23.0 ºC, and 22.7 - 91.2%, respectively).

3.2. Experimental Design

A total 65 lactating Friesian dairy cows in completely randomized design (CRD) were subjected to an estrus detection program by visual observation (twice per day) between day 25 to 32 (pp. If estrus was not observed by day 32, cows were rectally palpated to determine ovarian activity. Only cows with corpora lutea on rectal palpation were injected with 25 mg $PGF_{2\alpha}$ (Lutalyse; Pharmacia & Upjohn S.A., Puurs, Belgium) on day 32.

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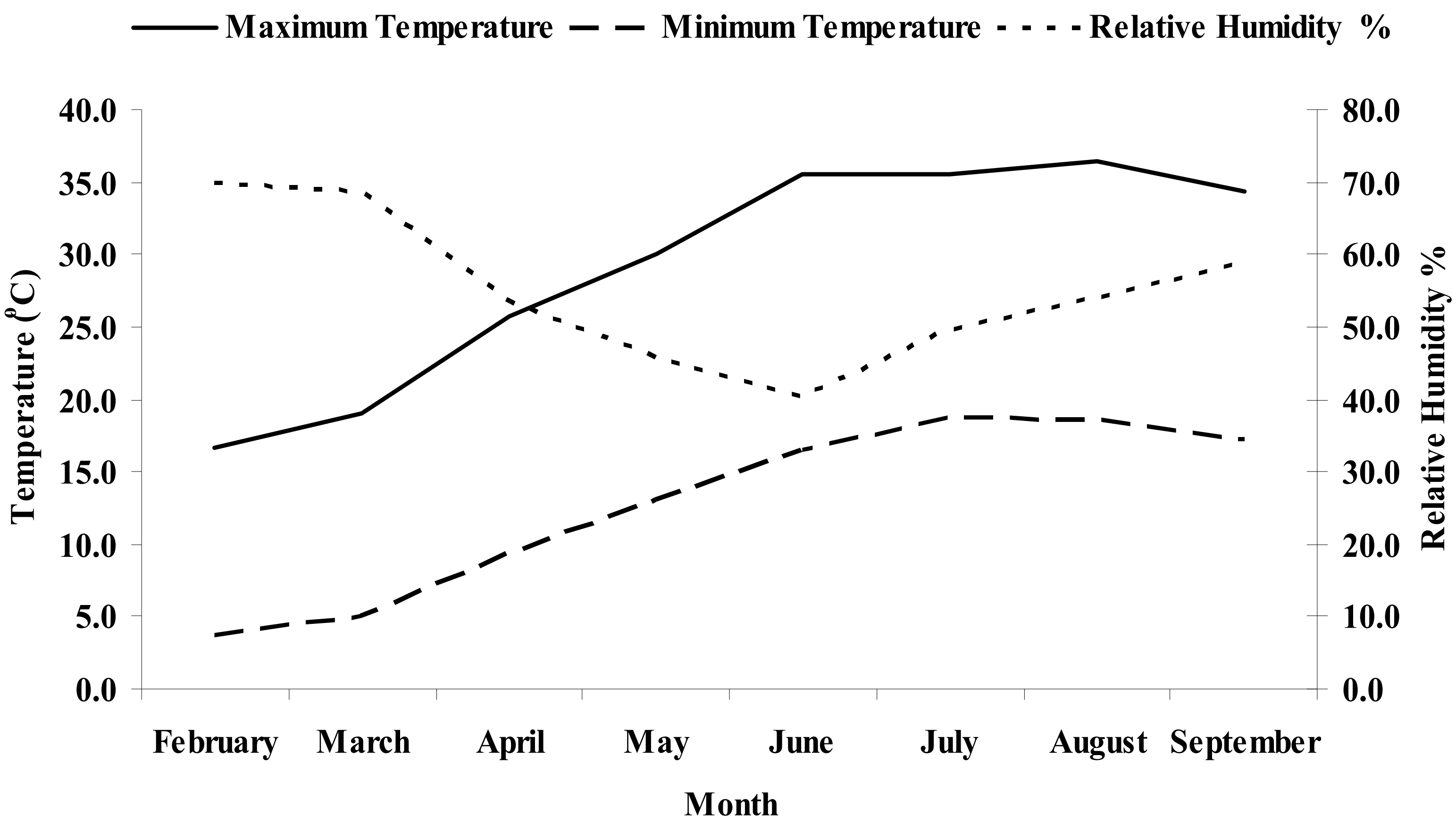


Figure 1. Trend of maximum, minimum temperature, and relative humidity during the months of the experimental period.

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Ten cows exhibited estrus between 28 and 32 day pp while twelve cows were excluded from the study because they either did not respond to $PGF_{2\alpha}$ injection on day 32 or due to different reasons such as diseases and culling. Therefore, only fifty-three cows (8 primiparous and 45 multiparous) were used in the study.

Ten to twelve days after the observed estrus (d 46 ± 3 pp), cows were randomly assigned into two treatments (Figure 2). Cows in the Ovsynch (Control) treatment (n = 24) received an i.m. injection of 10µg GnRH agonist (Buserelin, Receptal®, Hoechst Roussel Vet GmbH, Wiesbaden, Germany), followed 7 d later by an i.m. injection of 25 mg $PGF_{2\alpha}$ and 48 h later by a second i.m. injection of 10µg GnRH. The protocol was terminated with the timed artificial insemination (TAI) which took place 16-20 h after GnRH.

Cows in the Short Estrus Synchronization treatment (Short ES; n = 29) received an i.m. injection of 25 mg $PGF_{2\alpha}$ and an i.m. injection of 1 mg estradiol benzoate (EB) dissolved in oil (Intervet International B.V. Boxmeer, Holland) and 10µg GnRH 48 and 60 h after $PGF_{2\alpha}$; respectively. Later cows were inseminated artificially at detected estrus according to the AM-PM rule (12 h after standing heat).

Cows in both treatments were subjected to an estrus detection program by visual observation. One experienced AI technician using proven fertility semen (ABS Global, Inc., Deforest, Wisconsin, USA) performed AI throughout the experiment.

All cows were diagnosed for pregnancy on days 21, 22 and 23 (22±1 d), (first examination) after either TAI or AI based on P_4 concentrations. Progesterone concentrations equal to or exceeding 1 ng/ml were considered as an indication for a functional CL. Pregnancy was confirmed by rectal palpation between days 45 and 50 (second examination) after AI. Pregnancy losses were calculated as cows that were diagnosed pregnant at the first examination and diagnosed non-pregnant at the second

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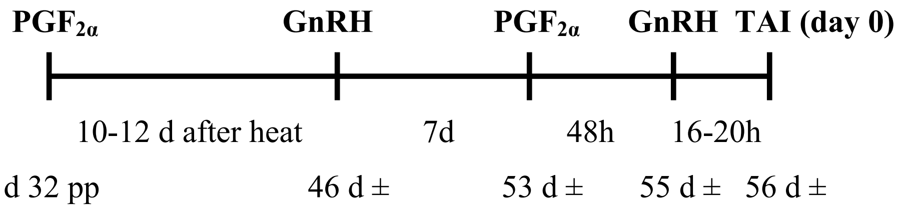
examination. Cows in both treatments, which were diagnosed not pregnant on day 22±1 or between 45-50 d received a new i.m. injection of 25 mg $PGF_{2\alpha}$ and inseminated after estrus detection with an i.m. injection of GnRH within 30 minutes until the fourth AI. Pregnancy rates were calculated for the two examinations and for the next three inseminations, while the overall pregnancy rates were defined as the percentage of cows that become pregnant after the fourth AI. Days from calving to first, first to second, second to third, and third to fourth AI were calculated. Days open were defined as the number of days from calving to conception for pregnant cows until the fourth AI while number of AI were calculated as number of AI for pregnant cows until the fourth AI.

3.3. Blood Sampling and Radioimmunoassay (RIA)

Blood samples were collected via the coccygeal venipuncture from all cows into heparinized tubes on days 0 (day of AI), 7, 14, 21, 22, and 23 after AI to determine P_4 concentrations. Plasma was harvested by centrifugation within 30 minutes at 3000 rpm for 15 minutes. Plasma samples were stored at -20 ºC until assayed for P_4 concentrations. Plasma P_4 concentration was determined by RIA (Immunotech a Beckman Coulter Company, Marseille, France) employing highly specific polyclonal antibodies in a radioimmunoassay for the quantitative determination of P_4 in plasma (Appendix A). The standards used for plasma were 0, 0.11, 0.5, 1.75, 9.6, and 60 ng/ml. The inter- and intra-assay coefficients of variation (CV %) were 9.0% and 5.8%, respectively.

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- **Ovsynch (Control, n = 24)**



- **Short ES (n = 29)**

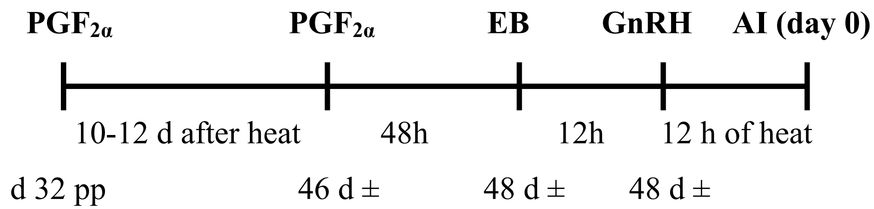


Figure 2. A schematic diagram of the hormonal treatments of lactating dairy cows in the study.

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3.4. Statistical Analysis

Statistical analysis was performed using the SAS (2000) to test the overall effects of factors. The effect of treatment (Ovsynch and Short ES) on estrus detection rate after hormonal treatment and pregnancy rate based on P_4 concentration at days 22+1 and by rectal palpation between 45-50 d post AI and pregnancy losses between the two diagnoses were tested by Chi-square test using the FREQ procedure of SAS.

Using General Linear Model (GLM) procedure of SAS, least square analysis of variance was applied on reproductive responses including effects of treatment (Ovsynch and Short ES), parity (primiparous and multiparous), and their two-way interactions. Responses included percentage of pregnancy rates at two diagnoses, the accumulated pregnancy rates up to the fourth AI, pregnancy losses between the two examinations, days from calving to first AI, first to second AI, and second to third AI, days open, and number of services per conception. Least square means for significant effects were compared at $P < 0.05$ using *t*-test Analysis of variance was performed to determine effects maximum temperature (from calving until 10 weeks) to first service and between treatments. In addition to that, the effect of plasma P_4 concentration and milk production for the first 20 weeks after parturition on treatments was estimated. All these responses were estimated using the FREQ procedure of SAS.

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4. RESULTS

The average daily milk production at the time of starting experiment did not differ ($P > 0.05$) between cows in the Ovsynch (36.9 ± 1.3 kg) and Short ES (38.9 ± 1.4 kg) treatments. Likewise, mean lactation numbers for cows in the Ovsynch (3.0 ± 0.26) and Short ES (2.6 ± 0.22) treatments were not significantly different.

4.1. Effect of Treatment and Parity on Estrus Detection Rate

Synchronization in Ovsynch and Short ES cows was initiated between 10 to 12 d of the estrous cycle. No cows in Ovsynch and Short ES treatments exhibited estrus prior the last hormonal injection. An effect ($P < 0.01$) of treatment but not parity on estrus detection rate was observed. On day 0 (day of AI), all cows in the Ovsynch treatment were inseminated and only 41.7% (10/24) of cows exhibited estrus within 24h following the second GnRH injection in the ovsynch group. In contrast, 15, 7, and 3 cows in the Short ES treatment exhibited estrus which is around 86.2% (25/29) and inseminated within 24, 48, and 72 h, respectively, after GnRH injection. Among those cows in both group observed in estrus, 20 % (2/10) and 40 % (10/25) of cows in the Ovsynch and Short ES treatments, respectively, become pregnant after the first AI.

4.2. Effect of Treatment and Parity on Pregnancy Rates and Pregnancy Losses

Pregnancy rates and pregnancy losses for cows based on treatment and parity are illustrated in Table 1. A greater pregnancy rate was observed by P_4 concentrations on d 22±1 post AI than that based on rectal palpation (45 d). Pregnancy rates at d 22±1 were similar for Ovsynch (54.2%) and Short ES (62.1%) cows. Pregnancy rates to first AI were lower ($P < 0.05$) for cows in the Ovsynch (12.5%) than those for cows in the Short ES (37.4%) treatments. In contrast, pregnancy rates to second,

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third, and overall were not different for Ovsynch and Short ES cows (table 1). Differences ($P < 0.01$) were observed in pregnancy rates based on P_4 concentrations on day 22±1 between primiparous (100%) and multiparious (51.1%) cows, while pregnancy rates to first (50.0 vs. 22.2%), second (75.0 vs. 48.9 %), third (75 vs. 57.8%), and overall (100 vs. 80%) were similar between primiparous and multiparous cows, respectively.

Differences were observed in pregnancy losses due to treatment effects, but not parity and treatment parity interaction. Pregnancy losses from 22±1 to 45d use after insemination were greater ($P < 0.05$) for cows in Ovsynch (76.9%) group than cows in the Short ES group (38.9%), but similar for primiparous (50.0%) and multiparous (56.5%) cows.

4.3. Effect of Maximum Temperature on Pregnancy Rates

The weekly maximum temperature did not differ between both treatments for the first 8 weeks after calving while it was greater ($P < 0.01$) for cows in the Short ES (34.25 ± 0.62 ºC) treatment than cows in the Ovsynch (29.43 ± 0.69 ºC) treatment at the second AI. At the third AI, the weekly maximum temperature did not differ between both treatments while it was greater ($P < 0.01$) for cows in the Ovsynch (37.05 ± 0.62 ºC) treatment than for those in the Short ES (34.3 ± 0.56 ºC) treatment at the fourth AI.

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Table 1. Pregnancy rates and pregnancy losses for cows according to treatment and parity.

Parameter	Treatment[1]		Parity	
	Ovsynch (n = 24)	Short ES (n = 29)	Primiparous (n = 8)	Multiparous (n = 45)
Pregnancy Rates	(n) %	(n) %	(n) %	(n) %
Based on Blood Sample at day 22±1[2]	(13) 54.2	(18) 62.1	(8) 100.0[a]	(23) 51.1[b]
First AI[3]	(3) 12.5[c]	(11) 37.9[d]	(4) 50.0	(10) 22.2
First and Second	(14) 58.3	(14) 48.3	(6) 75.0	(22) 48.9
First, Second, and Third	(17) 70.8	(15) 51.7	(6) 75.0	(26) 57.8
Overall[4]	(19) 79.2	(25) 86.2	(8) 100.0	(36) 80.0
Pregnancy Losses[5]	(10) 76.9[c]	(7) 38.9[d]	(4) 50.0	(13) 56.5

[1] Ovsynch (GnRH + $PGF_{2\alpha}$+ GnRH + TAI 16 to 20 h after second GnRH); Short ES ($PGF_{2\alpha}$+ EB + GnRH + AI at detected estrus).

[2] Based on plasma P_4 concentraton (> 1 ng\ml) on day 21, 22, and 23 post AI

[3]Cows that were pregnant 45 to 50 d after AI.

[4]Up to fourth AI.

[5]Percentage of cows diagnosed pregnant at day 22±1 after AI that were diagnosed non-pregnant between day 45 and 50 after AI.

[a, b]Percentages between treatments with different superscripts differ (P < 0.01).

[c, d]Percentages between treatments with different superscripts differ (P < 0.05).

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4.4. Effect of Treatment and Parity on Reproductive Performance Parameters

Reproductive performance parameters for cows based on treatment and parity are illustrated in Table 2. Treatment differences ($P < 0.01$) but not parity were detected for the interval from calving to first AI for cows in the Ovsynch (58.3 ± 1.2 d) and Short ES (47.3 ± 1.1 d) groups. These differences occurred due to experimental design. The intervals from first to second, second to third and third to fourth AI were not different for Ovsynch and Short ES cows. Similar observations in the same parameter were found between primiparous and multiparous cows, with the mean intervals between estruses approximately 28 ± 12 d, since all cows were observed for estrus twice daily by visual observation. Days open for pregnant cows tended to be higher ($P < 0.09$) for cows in Ovsynch (111.7 d) group than for cows in Short ES (74.7 d) group, while no differences were observed between primiparous (91.9 d) and multiparous (94.5 d) cows. The number of insemination per pregnancy was similar for Ovsynch and Short ES cows (2.6 ± 0.4 vs. 1.8 ± 0.4; respectively, table 2) and between primiparous and multiparous cows (2.1 ± 0.5 vs. 2.4 ± 0.2; respectively).

4.5. Milk Production for Cows According to Treatment and Pregnancy Status

The milk production for cows of the study according to the treatment and pregnancy status are shown in Figures 3 and 4. Milk production in the first 8 weeks pp did not differ between cows in the Ovsynch and Short ES treatments. Differences ($P<0.05$) were observed at weeks 9 (31.3 ± 2.0 vs. 38.1 ± 1.7 kg), 10 (32.7 ± 1.9 vs. 37.9 ± 1.6 kg), and 11 (32.5 ± 1.9 vs. 36.0 ± 1.7 kg), for

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cows in the Ovsynch and Short ES groups, respectively. Similar milk production was observed after that up to 20 weeks. In addition, milk production did not differ between pregnant and non- pregnant cows in the study.

4.6. Progesterone Concentrations for Cows According to Treatment and Pregnancy Status

Plasma progesterone (P_4) concentrations at days 0, 7, 14, 21, 22, and 23 after AI for cows in both treatments are illustrated in Figure 5. Plasma P_4 concentrations tended to be greater ($P < 0.1$) for cows in the Ovsynch (0.67 ng/ml) treatment than for those in the Short ES (0.44 ng/ml) treatment on day 0. In contrast, P_4 was greater ($P < 0.01$) for cows in the Ovsynch (7.38 ng/ml) treatment than for those in the Short ES (3.37 ng/ml) treatment on day 7 after AI. However, plasma P_4 concentrations did not differ between both treatments at days 14, 21, 22, and 23 after AI.

The effect of pregnancy status on plasma P_4 concentrations after first AI is illustrated in Figure 6. Plasma P_4 concentrations did not differ between pregnant and nonpregnant cows at days 0 and 7 after AI but it was greater ($P < 0.01$) for pregnant (9.08, 7.12, 7.34 and 8.33 ng/ml) than for nonpregnant cows (3.75, 2.98, 2.98 and 3.35 ng/ml) at days 14, 21, 22, and 23 after AI, respectively.

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Table 2. Reproductive performance for cows based on treatment and parity.

Parameter	Treatment[1]		Parity	
	Ovsynch (n = 24)	Short ES (n = 29)	Primiparous (n = 8)	Multiparous (n = 45)
Interval (d) from				
Calving to First AI	58.3 ± 1.2[a]	47.3 ± 1.1[b]	52.2 ± 1.4	53.4 ± 0.6
First to Second AI	42.7 ± 9.4	43.6 ± 14.8	53.5 ± 16.1	32.8 ± 5.0
Second to Third AI	19.0 ± 12.3	18.5 ± 13.1	11.8 ± 16.1	25.7 ± 5.5
Third to Fourth AI	15.8 ± 14	22.8 ± 14	10 ± 17.5	28.5 ± 6.6
Days Open[2]	111.7 ± 17.1[e]	74.7 ± 14.8[f]	91.9 ± 19.2	94.5 ± 8.7
Number of AI[2]	2.6 ± 0.44	1.8 ± 0.38	2.1 ± 0.49	2.4 ± 0.22

[1] Ovsynch (GnRH + $PGF_{2\alpha}$+ GnRH + TAI 16 to 20 h after second GnRH); Short ES ($PGF_{2\alpha}$+ EB + GnRH + AI at detected estrus).

[2]For pregnant cows.

[a, b]Percentages between treatments with different superscripts differ ($P < 0.01$).

[e, f] Percentages between treatments with different superscripts tended to be differ ($P < 0.1$).

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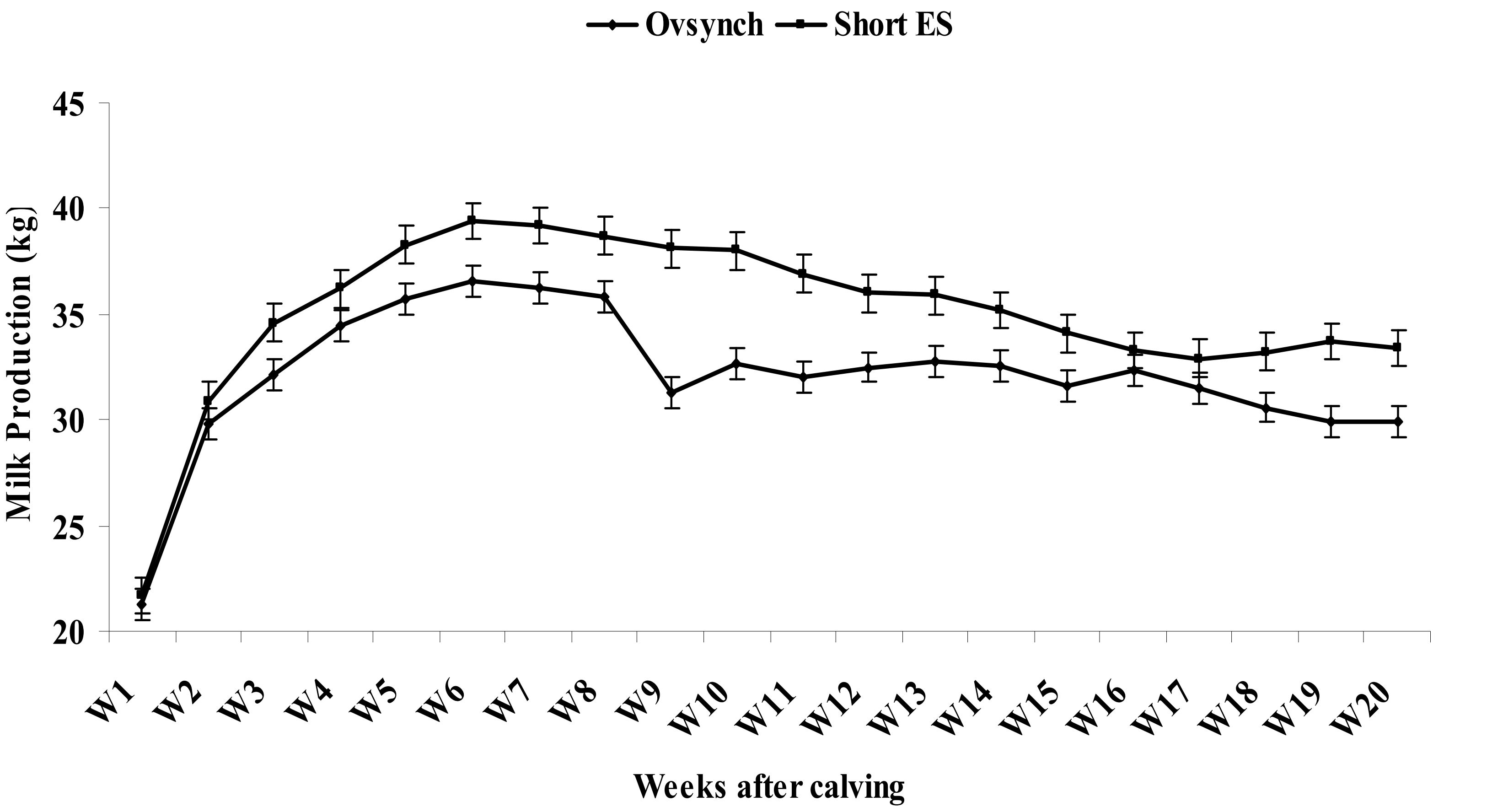


Figure 3. Weekly milk production for cows in the Ovsynch and Short ES treatments.

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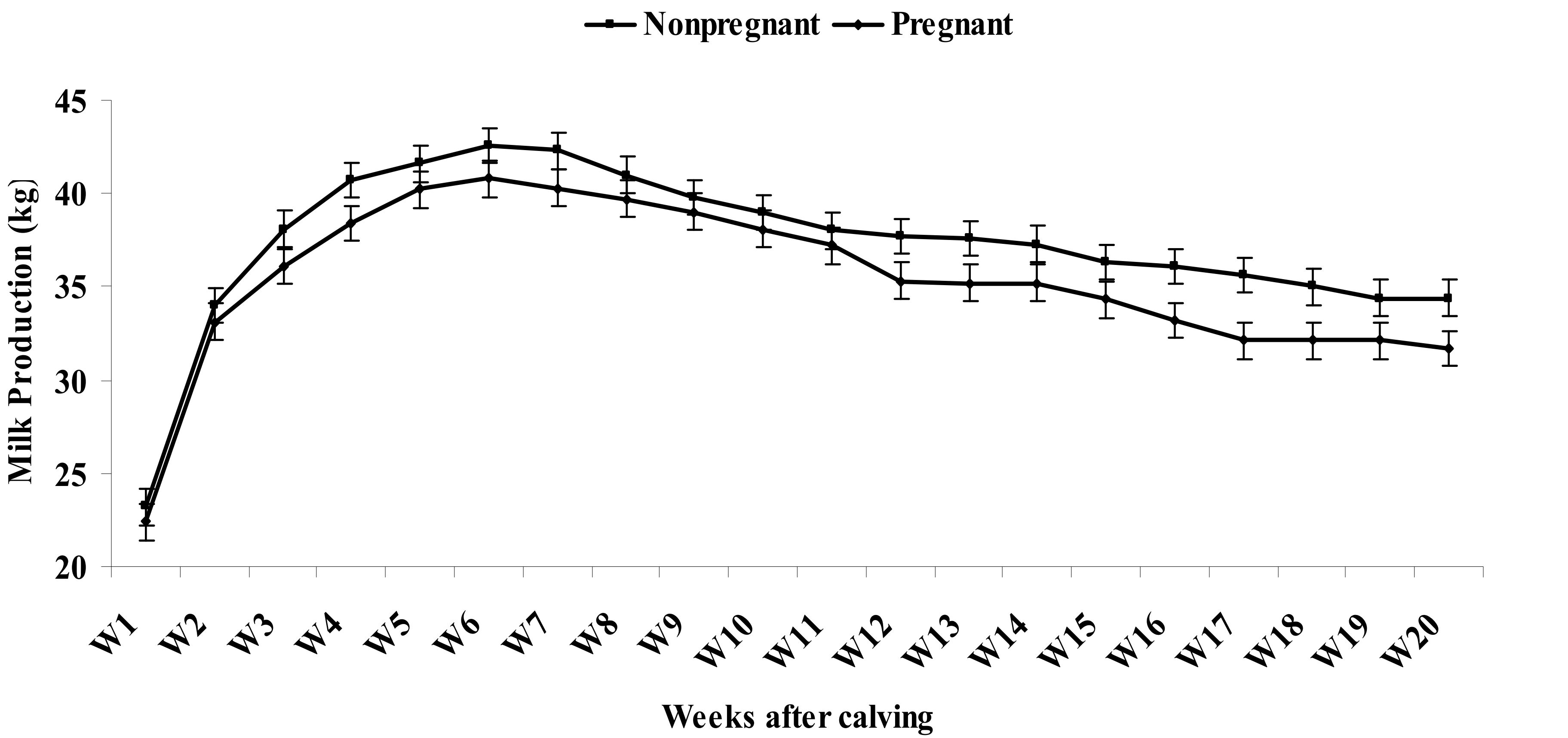


Figure 4. Weekly milk production for pregnant and nonpregnant cows.

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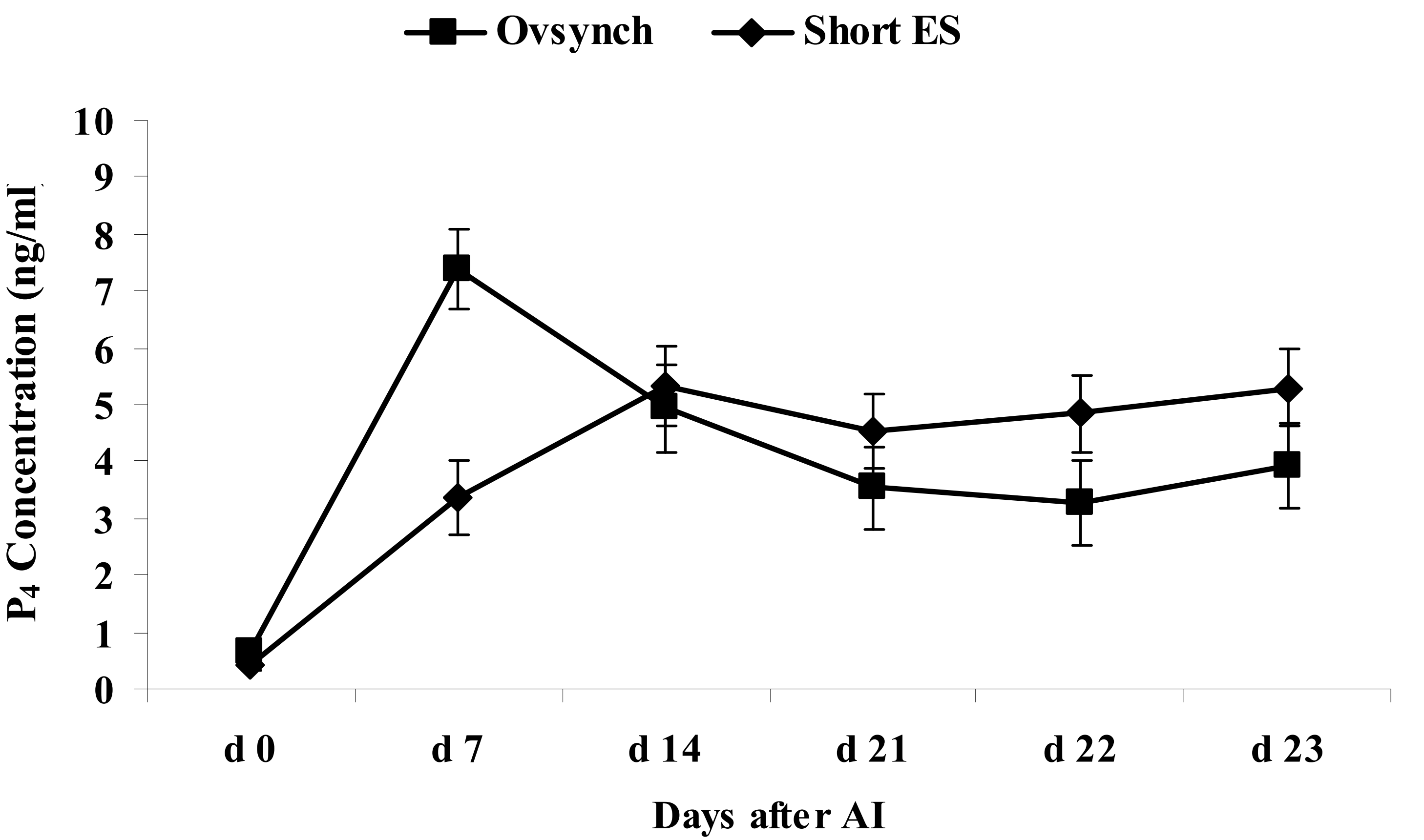


Figure 5. Plasma P_4 concentrations for cows in the Ovsynch and Short ES treatments after AI.

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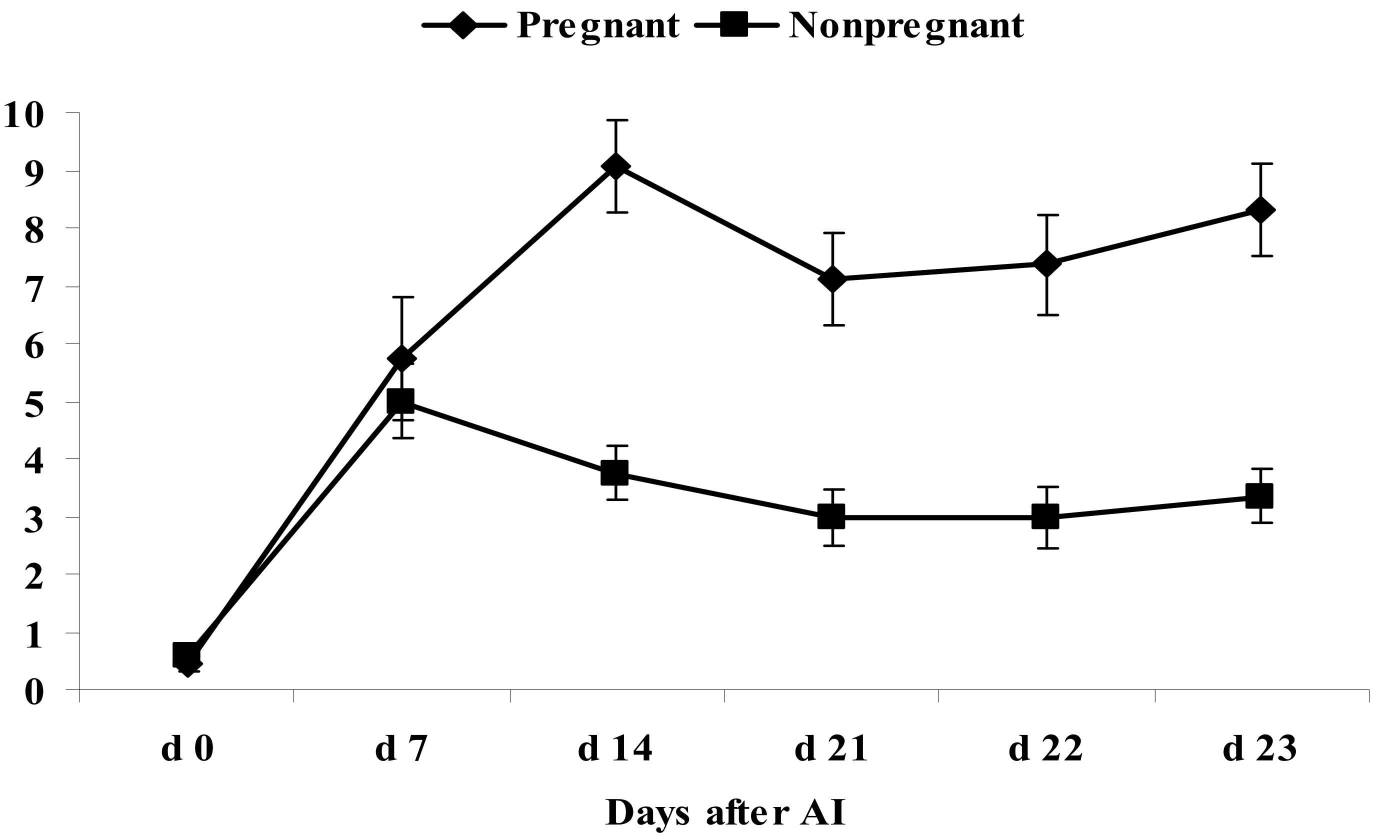


Figure 6. Plasma P_4 concentrations for pregnant and nonpregnant cows after AI.

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5. DISCUSSION

According to our knowledge, this study is the first conducted to compare the effect of two estrus synchronization protocols after pp on improving reproductive performance in dairy cows by using Ovsynch protocol or a Short ES involving $PGF_{2\alpha}$, E_2 and GnRH then inseminated at estrus detection. The pregnancy rate to first AI was significantly affected by treatment and parity. In addition, days from calving to first AI and days open were the only reproductive performance parameters that were affected by treatment. On the other hand, the average daily milk production in the first eight weeks after calving did not differ between treatments.

5.1. Effect of Treatment and Parity on Estrus Detection Rate

Synchronization with Ovsynch or Short ES was initiated between 10 to 12 d of the estrous cycle. No cows in both groups exhibited estrus prior to the last hormonal injection. Most previous studies reported around 10% (6% to 10.3%) or less cows observed in estrus before the final GnRH injection of the Ovsynch protocol (Mialot, et al., 1999; Stevenson, et al., 1999; Vasconcelos, et al., 1999; Alnimer, et al., 2009). However, Cirit, et al. (2008a) did not show in their study how many cows exhibited estrus before the end of short estrus synchronization protocol.

On day 0 (day of AI), all cows in the Ovsynch treatment were inseminated and around 42% of cows exhibited estrus within 24 h following the second GnRH injection. Souza et al. (2007) found that 44.4% of the cows in the Ovsynch protocol expressed in estrus 48 h after $PGF_{2\alpha}$ injection. On the other hand, 51.7, 24.1, and 10.3% of cows in the Short ES treatment exhibited estrus and inseminated between 24, 48 and 72 h following GnRH injection. Cirit, et al. (2008) reported that the rate of estrus in cows with an active CL was higher (72.7%) than those cows without an active CL (30.0%) at the start of a short synchronization program.

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In the present study, similar estrus detection rate was observed between primiparous and multiparous cows as previously cited by Souza, et al. (2007). In contrast, Hillegass. et al. (2008) found that primiparous cows were more likely to be detected in estrus than multiparous cows.

5.2. Effect of Treatment on Pregnancy Rates and Pregnancy Losses

A greater pregnancy rate was observed by P_4 concentrations on day 22±1 than that based on rectal palpation (45 d). These results are in agreement with those of Ababneh, et al. (2007), who found that pregnancy rates at 19 to 22 d post AI in lactating dairy cows range from 50 – 60%. The sustained increase in P_4 levels on day 22±1 post AI might represent established pregnancies that were lost before pregnancy diagnosis on day 45 post AI.

In the current study, pregnancy rates to first AI for cows in the Short ES group were similar (38%) than in the study of Cirit, et al. (2008a) who reported that pregnancy rates to first AI for cows in the Short ES timed AI protocol starting at diestrus of the cycle were around 33.3%. Moreover, pregnancy rates were greater in the Ovsynch protocol supplemented with E_2 for cows that showed estrus than those cows that did not show estrus (Souza, et al., 2007; Brusveen, et al., 2009). Unexpectly, in the present study, pregnancy rates in the Ovsynch protocol were lower than previous studies (Alnimer, 2005; Alnimer, et al., 2009), but the reason for that may be due to a tendency in P_4 concentrations for the Ovsynvh protocol at day 0 or due to some cows, which, were in heat before day 0 and did not showed estrus.

Pregnancy rates to second, third, and overall inseminations did not differ between both groups. Similar results were found in lactating dairy cows between Ovsynch and protocols involving GnRH, $PGF_{2\alpha}$ and E_2 (Alnimer, 2005) or Ovsynch supplemented with E_2 (Brusveen, et al., 2009).

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Differences were observed in pregnancy losses due to treatment effects. Pregnancy diagnosis on day 22±1 was measured by P_4 concentrations but not by ultrasound. Portaluppi and Stevenson (2005) reported that the highest pregnancy losses were observed in the Ovsynch protocol than other TAI protocols. In contrast, Lubbadeh and Alnimer (2003) and Ababneh, et al. (2007) reported similar pregnancy losses between first diagnoses by P_4 concentrations and rectal palpation. In present study, the higher pregnancy losses in the Ovsynch protocol may be due to some cows had delayed luteolysis (cycle > 23 d long).

5.3. Effect of Parity on Pregnancy Rates and Pregnancy Losses

Differences were observed in pregnancy rates based on P_4 concentrations on day 22±1 between primiparous and multiparous cows. Balendran, et al. (2008) reported that pregnancy rates based on P_4 concentrations from d 0 to d 22 post AI among first parity cows were higher than third and fourth parity cows.

Primiparous and multiparous cows were similar in pregnancy rates from first until overall and pregnancy losses regardless of the treatment. Jobst, et al. (2000), Navanukraw, et al. (2004), and Alnimer (2005a) reported similar pregnancy rates between primiparous and multiparous cows. In contrast, Tenhagen, et al. (2004), Chebel, et al. (2004), Brusveen, et al. (2009), Souza, et al. (2007), Alnimer, et al. (2009), and Santos, et al. (2009) found greater pregnancy rates and lower pregnancy losses for primiparous cows than multiparous cows. It is well documented that pregnancy rates decrease with increasing in lactation number (Peters and Pursley, 2003; Grimard, et al., 2006; Balendran, et al., 2008). There are conflicting reports about differences in pregnancy rates between primiparous and multiparous cows. These differences may be explained by the use of different breeds, drugs, nutritional status, body condition score, and time of initiation of the protocol (Grohn and Rajala-Schultz,

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2000). Moreover, Huszenicza et al., (1987) reported that primiparous cows had fewer reproductive problems compared to older cows.

5.4. Effect of Maximum Temperature on Pregnancy Rates and pregnancy losses

Mean maximum temperature from calving until first AI between both groups was not significant; therefore, the differences in pregnancy rates between two groups may be due to the treatment effects. On the other hand, pregnancy rates at the second AI in the Ovsynch group were numerically higher than in the Short ES group; this may be due to a higher maximum temperature at the time when cows of Short ES were inseminated. In contrast, pregnancy rates at fourth AI in the Short ES group were numerically higher than in the Ovsynch group. Al-Katanani, et al. (2002) reported that heat stress before and after breeding, and on the day of breeding was associated with low pregnancy. In addition, heat stress had a delayed negative effect on pregnancy rates up to 50 d before AI (Roth et al., 2001a & b; Chebel et al., 2004). Previous studies reported lower pregnancy rates and greater pregnancy losses for cows inseminated in summer than those for cows inseminated in winter (Roth et al., 2001a & b; Chebel et al., 2004).

5.5. Effect of Treatment and Parity on Reproductive Performance

The intervals between inseminations from first until fourth, and number of AI per pregnancy did not differ between groups and parity, but a tendency was found in days open between both groups (Table 2). Because pregnancy rates to first AI were lower for cows in the Ovsynch treatment than for cows in Short ES treatment, additional inseminations were needed for cows in the Ovsynch treatment to become pregnant, with a tendency higher in days open. This was primarily related to the greater pregnancy rates to first AI for cows in the short ES treatment than those for cows in the Ovsynch treatment while cows in the short ES treatment were inseminated shorter than other

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treatment by about 11 d due to experimental design. Moreover, mean days interval between estruses was around 28 d. In practice, the return to estrus is variable with most cows returning to estrus 20 to 24 d after insemination (Chenault, et al., 2003). The variability in return to estrus may be explained by the normal variation in estrus length and early embryonic death or due to high environmental temperature from the second AI until the end of experiment. Heat stress is associated with a decreased expression of estrous behavior (Nebel, et al., 1997) and increases in the percentage of undetected estrus (Thatcher and Collier, 1986). The consequence of these changes is a reduction in the number of inseminations and an increase in the proportion of inseminations that do not result in pregnancy (Hansen, 2007).

5.6. Effect of Milk Production on Pregnancy Rates and Pregnancy Losses

Average of milk production from calving to around first AI was similar between both groups while three weeks later milk production was higher for Short ES group than Ovsynch group. This may explain why pregnancy rates at second and third AI were higher numerically in the Ovsynch group than in the Short ES group. Regardless of treatment, overall pregnancy rates were lower than previous studies (Lubbadeh and Alnimer, 2003; Alnimer, 2005b). This may be due to a higher milk production for cows in the present study as reported by Lucy (2001). In addition, cows are in NEB after calving and milk production gets preference over the other physiological processes including reproduction, thus, anovulatory anestrus period may extended (Stevenson, et al., 1997; Mwaanga and Janowski, 2000; Grimard, et al., 2006). Moreover, the reproductive efficiency of the dairy cows has decreased in the past two decades with an increase in milk production, which is related to the prolonged NEB (Grohn and Rajala-Schultz, 2000; Roche, et al., 2000, Washburn, et al., 2002).

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5.7. Progesterone concentrations for Cows According to Treatment and Pregnancy Status

Plasma P_4 concentrations tended to be higher for cows in the Ovsynch treatment than for those in the Short ES treatment at day of AI. This means that the CL of some cows did not regress completely or due to the start of new development of the CL, Therefore, the low pregnancy rate in the Ovsynch group may be due to the greater of P_4 concentrations on day of AI since this protocol utilizes TAI without heat detection except for cows observed in heat at that time. A higher P_4 concentration in the Ovsynch treatment, was due to some cows, which, were in heat before day 0 and did not exhibited estrus.

Plasma P_4 concentrations were significantly higher in pregnant than nonpregnant cows on day 14 in the present study. Similar findings were reported by Herzog, et al. (2011). In a previous study, peripheral P_4 concentrations were higher in pregnant than nonpregnant cows as early as day 10 (Hansel, 1981). In contrast, it was reported that P_4 concentrations were not significantly different between pregnant and nonpregnant inseminated cows from 7 to 15 d after insemination (Echternkamp, et al., 2009). These apparent differences may be attributed to varying numbers of animals per study, management factors, and P_4 assays.

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6. CONCLUSIONS AND RECOMENDATIONS

Short ES protocol improves reproductive performance by increasing number of cows observed in estrus and pregnancy rates to first AI than Ovsynch protocol. In contrast, pregnancy losses were higher in the Ovsynch protocol than in the Short ES protocol. Pregnancy rates to second, third, and overall were similar between both groups and between primiparous and multiparous cows. Further studies with higher number of animals are needed to investigate the lack of pregnancies at first AI in the Ovsynch protocol.

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8. APPENDICES

8.1. Appendix A: Progesterone Radioimmunoassay

8.7.1. Principle of the Assay

The Coat-A-Count procedure (Immunotech, Marseille, France) is a solid-phase ^{125}I radioimmunoassay designed for the directed, quantitative measurement of P_4.

Labeled P_4 competes for a fixed time with P_4 in serum sample for antibody sites. Because the antibody is immobilized to the wall of polyprophlene tube, decanting the supernatant suffices to terminate the competition and to isolate the antibody bound fraction of the radio labeled P_4. Counting the tube in a gamma counter then yields a number, which converts by way of a standard curve to measure P_4 found in the sample by interpolation.

8.7.2. Sample Collection

Blood samples (5 ml) were collected from the cow via the coccygeal venipuncture into lithium heparin vacutainers and centrifuged (3000 rpm for 15 min.) within 30 min. of collection. Plasma was harvested using plastic pastuer pipettes into plastic tubes and stored frozen at – 20 °C until assayed for P_4 (Immunotech, Marseille, France).

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8.7.3. Procedure

Note: all components of the kit and sample must be at room temperature before use.

- Label the 14 tube for standards as shown in the following table:

Label	Tube	P_4 concentrations (ng/ml)
TC	Plain (uncoated)	-
TC	Plain (uncoated)	-
MB (S0)	Coated	0
MB (S0)	Coated	0
S1	Coated	0.11
S1	Coated	0.11
S2	Coated	0.5
S2	Coated	0.5
S3	Coated	1.75
S3	Coated	1.75
S4	Coated	9.6
S4	Coated	9.6
S5	Coated	60.0
S5	Coated	60.0

- Label coated tubes for blood serum samples.
- Pipette 50µl of each of the calibrators (0-5) and blood serum sample into corresponding tubes. All pipetted calibrators and samples must be vortexed before pipetting.
- Pipette 500µl of tracer (^{125}I-progesterone) into all tubes.

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- Pipetting of tracer into a set of tubes (rack) must be done within 10 min.
- Vortex all tubes carefully.
- Incubate all tubes at (18-25 °C) with shaking (350 rpm) for 60 min.
- Decant the content of all tubes thoroughly, except of the tube of the total count (TC).
- Count all tube for 1 min in a gamma counter.

8.7.4. Reading Interpolation

Standards of the assay are plotted against the percent binding of the labeled P_4 in a logit-log graph. Percent binding of all unknown samples is then calculated and plasma P_4 concentrations are extracted from the graph by interpolation.

Alternatively, all tube can be read on DPC-C12 gamma counter, LA. In this situation, concentrations of standards are plugged into the software interface of the counting control interface. The output of the interpolation is then performed automatically. The results of P_4 concentrations are then printed out readily.

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مقارنة استخدام برنامجين لتنظيم الشياع على الأداء التناسلي لأبقار الحليب

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ملخــص

أجري هذا البحث لمقارنة برنامجين لتنظيم الشياع على الأداء التناسلي لأبقار الفريزيان الحلابة، تم إستخدام 53 بقرة بعد الولادة وتحديداً ما بين اليوم 10-12 من دورة الشياع حيث قسمت الى مجموعتين: (1) Ovsynch (الشاهد، 24 بقرة) حقنت بهرمون المحفز للنخامية (GnRH) في اليوم 46 بعد الولادة والبروستاجلاندين ($PGF_{2\alpha}$) بعد 7 أيام وGnRH بعد يومين ومن ثم التلقيح الإجباري ما بين 16-20 ساعة بعد الجرعة الثانية من GnRH، (2) Short ES (29 بقرة) حقنت بهرمون $PGF_{2\alpha}$ في اليوم 46 بعد الولادة وبهرمون الإستراديول (E_2) بعد 48 ساعة ومن ثم بهرمون GnRH بعد 12 ساعة والتلقيح بعد إظهار الشياع. مراقبة الشياع كانت بالمشاهدة. تم أخذ عينات دم من جميع الأبقار في الأيام 0، 7، 14، 21، 22، واليوم 23 بعد التلقيح وذلك لتقدير هرمون البروجستيرون. تم تحديد نسبة الحمل عن طريق عينات الدم في الأيام 22±1 والتاكيد عليها بواسطةالجس اليدوي مابين 45-50 بعد التلقيح. ظهر الشياع في عدد كبير وبفرق معنوي ($P<0.01$) في أبقار مجموعة Short ES (86.2%) مقارنة بمجموعة Ovsynch (41.7%). أيضاً كانت نسبة الحمل من التلقيحة الأولى أعلى ($P<0.05$) في أبقار مجموعة Short ES (37.4%) مقارنة بمجموعة Ovsynch (12.5%) بالمقابل كانت نسبة فقد الأجنة أقل معنوياً ($P<0.05$) في أبقار مجموعة Short ES (38.9%) مقارنة بمجموعة Ovsynch (76.9%) . لم تكن هنالك فروق معنوية في نسبة الحمل من التلقيحة الثانية الى الكلية بين المجموعتين أو بين الأبقار ذات الولادة الواحدة أو متعددة الولادات. أما بالنسبة الى الأيام المفتوحة فلها ميل بالزيادة بالنسبة الى أبقار مجموعة Ovsynch مقارنة بمجموعة Short ES. تراكيز هرمون البروجستيرون كان متشابة في الأبقار الحوامل عن غير الحوامل في اليومين 0 و7 من التلقيح ولكن كانت التراكيز أعلى في الأبقار الحوامل مقارنة بغير الحوامل في الأيام 14، 21، 22 و23 بعد التلقيح. دلت نتائج هذة التجربة الى أن برنامج Short ES أظهر تحسناً في الأداء التناسلي للأبقار وذلك عن طريق مشاهدة أكبر عدد منها في حالة شياع بالإضافة الى تفوق في نسبة الحمل من التلقيحة الأولى بالمقارنة مع برنامج Ovsynch.

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